

EPO - DG 1

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CLAIMS

ATTACHMENT #1
ORIGINAL CLAIMS 1-Z4
FOR 0989078Z

I. Method of modulating in a cell the level(s) of one or more metabolites, and/or of modulating the expression of one or more genes involved in the biosynthesis of a metabolite or a precursor therefor, said method comprising providing to the cell an AP2-domain transcription factor that is involved in the response of a plant cell to a

jasmonate, or a variant of the AP2-domain transcription factor comprising at least one AP2-domain having an amino acid sequence with at least 40% amino acid identity with an AP2-domain of SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6.

2. A method according to claim 1 wherein the provision of the AP2-domain transcription factor or variant thereof results in a modulation of the stress resistance of a cell.

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3. Method according to claims 1 or 2, wherein the AP2-domain transcription factor or variant thereof is provided to the cell by the expression in said cell, under the control of an expression regulating sequence operable in said cell, of a nucleotide sequence that encodes the AP2-domain transcription factor or variant thereof.

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- 4. Method according to claim 3, comprising the steps of:
- (a) transforming the cell with a genetic construct, said construct comprising the nucleotide sequence encoding the AP2-domain transcription factor or variant thereof, operably linked to said expression regulating sequence;
- 25 (b) maintaining the cell under conditions such that the nucleotide sequence is expressed in said cell.
 - 5. Method according to claims 3 or 4, in which the expression regulating sequence is heterologous to the cell and/or in which the expression regulating sequence is an expression regulating sequence with which the nucleotide sequence that encodes the AP2-domain transcription factor or variant thereof is not natively associated.



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6. Method according to any of the preceding claims, wherein the cell to which the AP2-domain transcription factor or variant thereof is provided is a plant cell.

- 7. Method according to any of the preceding claims, wherein the plant cell is present in a plant, such as in a tissue, part or organ of a plant, or in a culture of plant cells, in which said plant or plant cells has/have been transformed with a genetic construct comprising a nucleotide sequence encoding the AP2-domain transcription factor or variant thereof operably linked to an expression regulating sequence operable in said plant or plant cells.
- 8. Method according to any of the preceding claims, wherein the at least one metabolite is a plant metabolite, including but not limited to precursors and/or intermediates therefor.

9. Method according to any of the preceding claims, wherein the plant metabolite is a secondary plant metabolite selected from the group consisting of alkaloid compounds, phenolic compounds or tempenoid compounds such as a terpenoid indole alkaloid.

- 10. Method according to any of the preceding claims, wherein the gene involved in the biosynthesis of the metabolite encodes a protein or polypeptide, including but not limited to an enzyme.
- 25 11. Method according to any of the preceding claims, in which, in the cell to which the AP2-domain transcription factor or variant thereof has been provided:
 - the level in the cell of at least one metabolite is enhanced by at least 10%, at least 25% or at least 100%, relative to a cell to which the transcription factor or variant thereof is not provided; and/or
- 30 the level in the cell of the at least one metabolite is reduced by at least 10%, at least 25% or at least 50%, or at least 95%, relative to a cell to which the transcription factor or variant thereof is not provided.

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- 12. Method according to any of the preceding claims, in which, in the cell to which the AP2-domain transcription factor or variant thereof has been provided:
- the expression in the cell of one or more genes involved in the biosynthesis of a metabolite or a precursor therefor is enhanced by at least 10%, at least 25% or at least 100%, relative to a cell to which the transcription factor is not provided; and/or
- the expression in the cell of one or more genes involved in the biosynthesis of a metabolite or a precursor therefor is reduced by at least 10%, at least 25% or at least 50%, or at least 95%, relative to a cell to which the transcription factor is not provided.
- 13. Plant, plant cell or plant material that has been transformed with a genetic construct comprising a nucleotide sequence encoding an AP2-domain transcription factor that is involved in the response of a plant cell to a jasmonate, or encoding a variant of the AP2-domain transcription factor comprising at least one AP2-domain having an amino acid sequence with at least 40% amino acid identity with an AP2-domain of SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6.
- 14. Plant, plant cell of plant material according to claim 13 that has a modified stress resistance relative to an untransformed plant, plant cell or plant material.
 - 15. Plant, plant cell or plant material according to claim 13 or 14, in which the expression regulating sequence is heterologous to the plant or plant cell and/or in which the expression regulating sequence is an expression regulating sequence with which the nucleotide sequence is not natively associated.
 - 16. Plant, plant cell or plant material according to any of claims 13-15, in which
 - the level in the plant, plant cell or plant material of at least one metabolite is enhanced by at least 10%, at least 25% or at least 100%, relative to the corresponding non-transformed plant, plant cell or plant material;
 - the level in the plant, plant cell or plant material of the at least one metabolite is reduced by at least 10%, at least 25% or at least 50%, or at least 95%, relative to the corresponding non-transformed plant, plant cell or plant material.

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17. Plant, plant cell or plant material according to any of claims 13-16, in which:

- the expression in the cell of one or more genes involved in the biosynthesis of a metabolite or a precursor therefor is enhanced by at least 10%, at least 25% or at least 100%, relative to the corresponding non-transformed plant, plant cell or plant material; and/or
- the expression in the cell of one or more genes involved in the biosynthesis of a metabolite or a precursor therefor is reduced by at least 10%, at least 25% or at least 50%, or at least 95%, relative to the corresponding non-transformed plant, plant cell or plant material.
- 18. Plant, plant cell or plant material according to any of claims 13-17, wherein the plant metabolite is a secondary plant metabolite selected from the group consisting of alkaloid compounds, phenolic compounds or terpenoid compounds such as a terpenoid indole alkaloid.
- 19. Plant, plant cell or plant material according to any of claims 13-18, wherein the gene product involved in metabolite production is a protein or polypeptide, including but not limited to an enzyme.
- 20. Cultivating material, such as seed, tubers, roots bulbs and/or seedlings, for or of transformed plant according to any of claims 13-19; plant material obtained from a plant of any of claims 13-19; or a descendant of a transformed plant according to any of claims 13-19, characterized in that the cultivating material, the plant material, or the descendant contains a genetic construct comprising a nucleotide sequence encoding an AP2-domain transcription factor that is involved in the response of a plant cell to a jasmonate, or encoding a variant of the AP2-domain transcription factor comprising at least one AP2-domain having an amino acid sequence with at least 40% amino acid identity with an AP2-domain of SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6.

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- 21. Method for providing a transformed plant or a descendant of such a transformed plant, in which, compared to the corresponding native non-transformed plant,
- the intracellular level(s) of one or more metabolites are enhanced, the intracellular levels of one or more (other) metabolites are reduced; or a combination thereof, e.g. as described above and/or by the amounts indicated above; and/or
- the expression of one or more genes involved in the biosynthesis of a metabolite or a precursor therefor are enhanced; the expression in the cell of one or more genes involved in the biosynthesis of a metabolite or a precursor therefor are reduced; or a combination thereof, e.g. as described above and/or by the amounts indicated above;

said method comprising the steps of:

- (a) transforming a plant, plant cell or plant material with a genetic construct comprising at least one nucleotide sequence encoding an AP2-domain transcription factor that is involved in the response of a plant cell to a jasmonate, or encoding a variant of the AP2-domain transcription factor comprising at least one AP2-domain having an amino acid sequence with at least 40% amino acid identity with an AP2-domain of SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6, whereby the nucleotide sequence is operably linked to an expression regulating sequence;
- (b) optionally cultivating the plant, plant cell or plant material into a mature plant; and/or
- (c) optionally providing one or more further generations of the transformed plant of step (a) and/or step (b) by sexual or asexual reproduction, including but not limited to standard plant cultivation and/or plant breeding techniques, whereby the further generations of the transformed plant contain a genetic construct comprising at least one nucleotide sequence encoding an AP2-domain transcription factor that is involved in the response of a plant cell to a jasmonate, or encoding a variant of the AP2-domain transcription factor comprising at least one AP2-domain having an amino acid sequence with at least 40% homology to AP2-domain of SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6.

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- 22 Method of producing a metabolite, the method comprising providing a recombinant cell or plant according to any of claims 13-19, cultivating said cell or plant under conditions conducive to the expression of the nucleotide sequence coding for AP2-domain transcription factor or variant thereof, whereby expression of the transcription factor or variant thereof causes the expression of at least one gene involved in the production of the metabolite, and recovering the metabolite from the plant cell, the plant, and/or plant material obtained from said plant.
- 23. A method according to claim 22 whereby the metabolite is a plant secondary 10 metabolite.
 - 24. A nucleit acid molecule comprising a nucleotide sequence selected from:
 - (a) SEQ ID NO: 1 SEQ ID NO: 2, and SEQ ID NO: 3;
 - (b) a nucleotide sequence encoding an AP2-domain transcription factor that is involved in the response of a plant cell to a jasmonate; and
 - (c) a nucleotide sequence encoding a variant of the AP2-domain transcription factor comprising at least one AP2-domain having an amino acid sequence with at least 40% homology to AP2-domain of SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6.



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